Morphological and Histochemical Studies on the Tegument and Parenchyma of Fasciola gigantica (Cobbold, 1855) and Observations on it's Lipid and Glycogen Contents

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This is to certify that the dissertation entitled " Morphological and Histochemical Studies on the Tegument and Parenchyma of Fasciola gigantica (Cobbold,1855) and observations on it's Lipid and Glycogen contents" being submitted in partial fulfilment of requirement of the M.Phil. M. Phil degree of Aligarh Muslim University is a piece of original work done by Mr. Ahmad Zaman under my supervision.

The work is suitable for the purpose and I permit Mr. Ahmad Zaman to submit it for the same.

(Hisamuddin Farooqi)

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INTRODUCTION

Fasciola gigantica Cobbold, 1855, the giant liver fluke of cattle is one of the most important Trematode parasites of Live-stock in the agricultural tropics and is of considerable a importance from patho-biological as well as economic points of view because it renders tons of beef liver useless and costs much to the cattle industry.

Except a few epizootological studies a country-wide epidemiological data of the incidence of this parasite is still wanting and quantum of financial loss to the cattle industry of India is yet to be ascertained.

This fluke is common in tropical and subtropical countries and happens to have remarkably high incidence in agricultural countries. The incidence appears equally high in India but surprisingly little work has been done on this parasite. In this country high incidence has been reported in many states, viz., Bihar, Orisa, Punjab and Uttar Pradesh, and Roy (1954) has reported 75% incidence in the cattle of Kalimpong (Sikkim).

In some countries the area of distribution overlaps with that of Fasciola hepatica. Such types of mixed infections have been reported from Pakistan (Kendall, 1954), Thailand (Disshmarn, 1955), Turkmenia, U.S.S.R. (Kibakin, 1961) and Japan (Watanabe, 1958). Such reports

are not available for India.

Besides causing Liver-rot or fascioliasis, F. gigantica is also responsible for causing " Black disease " in association with the bacterium, Clostridium oedematiens, which produces serious pathological syndrome in cattle. Every year many human cases are also reported.

At Aligarh abeatoir where buffaloes are brought mostly from adjoining areas, through a random fortnightly-survey conducted from April 1974 through December 1976 the incidence of F. gigantica was found to average 3% on the higher side, ranging between 6.6% (Sept. 76) and 7.23% (Jan. 76) whereas low parameters usually ranged between 3 & 4% with still low figures in certain months (Table I). However, what seems more important from the patho-biological point of view is the worm-burden in an infected host rather than the number of the cattle heads infected in a sample.

With augmented irrigational facilities and increasing snail populations fascioliasis is likely to become more wide-spread and serious a problem in the Plains of Uttar Pradesh and may take to the same pattern as has been reported by Patnaik (1971) from Orissa.

The present study deals with priliminary morphological and histochemical investigations related to the tegument and

parenchyma of *F. gigantica*, whereas, studies on other systems will follow.

MATERIAL AND METHODS

Live specimens obtained from the common bile ducts and gall bladders of infected hosts were first washed and brought to the laboratory in Hedon fleig's solution (Clegg, 1957) in Thermos containers. These were subsequently fixed in neutral formalin 10%, formalin 4%, Helley's fluid and Bouin's and also in Carnoy's fixatives for morphological and histochemical studies. Paraffin-processed material, sectioned at 5-8 μ were stained with H & E, Mallory's triple stain and Heidenhain's Azan. To to mounts were stained with Greenacher's Borax Carmine.

For qualitative histochemical studies thefollowing techniques were utilised:

1. For glycogen : PAS
2. " " : Best's Carmine
3. " Acid-mucopolysaccharides: Alcian blue
4. " Proteins : Mercuro-bromophenol blud
5. " Lipids : Sudan Black 3
6. " Bound lipids: Acetone-Sudan black.

Controls for glycogen localization were digested with α -amylase.

For quantitative glycogen estimation, 20 samples of known weight (0.5 gm. each) were first extracted from tissue

homogenate (Ashman & Seed, 1973) and determined through Spectronic-20 (Montgomery, 1957).

For determinating the lipid contents the conventional method of Soxhlet extraction through Petroleum-ether was prefered over other solvents like ethanol-ether extraction because the latter dissolves substances other than lipids as well. Ten samples of selected healthy flukes of known weight (2.5 gms. each) were first oven-dried and then subjected to extraction.

HISTORICAL REVIEW

Fasciola gigantica was first described by Cobbold (1855) from the liver of Giraffa camelopardalis and was subsequently referred to as Cladocoelium giganteum by Stossich (1892). Jackson (1921) reviewed the various species and gave a comprehensive account of the gross morphology of various forms. Varma (1953) also studied this parasite and described an identical form from the liver of goat and buffaloes and named it as Fasciola indica but Sarwar (1957) ultimately regarded it as a junior synonym of F. gigantica and this view is now widely held.

Thapar and Tandon (1952) elucidated the life-history of this parasite at Lucknow and found Lymnaea acuminata and L. auricularia as the common intermediate hosts. In recent years Patnaik (1971) furnished a comprehensive account of the autecology and synecology of this parasite as well as its intermediate hosts in Orissa.

Inspite of wide destribution in tropics and subtropics, little work seems to have been done on it's morphology. Except a few morphological studies like those of Bhalerao (1935), Rao and Madhavi (1962), Watanabe and Iwata (1955a, 1956c, 1958a); and Watanabe and Ueno (1959a, 1960a,b) on the anatomical features of it's larval forms, extensive

studies on the morphology of F. gigantica are still wanting. Most of the studies are either epizootological, ecological or empirical and quantitative or related to it's chemotherapy.

F. hepatica, being the commonest and most abundantly available fluke attracted attention of earlier workers and became an ideal tool and model of trematode morphology and physiology. Pioneer studies by early workers in this regard are those of Thomas (1881, 1882a,b; 1883a,b) and Leuckart (1881 & 1886); Weinland & Von Brand (1926) first set pace on histochemical and physiological aspects and elucidated the localization of glycogen in such parasites. Von Brand & Mercado (1961) elaborated such studies and these were followed by Pantelouris (1964b), and Halton (1967d).

On F. gigantica preliminary physiological studies have been made by Goil (1958a, b; 1961) on carbohydrate, protein and lipid metabolism, and, in recent years notable contribution have been made by Siddiqi & Lutz (1966) on it's ionic and osmotic regulation and Lutz & Siddiqi (1967) on the nature of haemoglobin of this parasite vis- a - vis the same moiety of it's host.

Compared to F. gigantica, much work has been done on it's closest congener, F. hepatica and has formed as the basis of generalization of trematode morphology and in certain respects, trematode physiology, notable among these being the studies of Prenant (1922), Miller (1923), Bugge (1929), Stephenson (1947); and Dawes (1954, 1962c; and 1963a).

The advent of electron microscopy, and more recently that of scanning electron microscopy have opened new vistas in morphology and many workers utilized these tools in studying various aspects of morphology, and, among other structures, tegument and parenchyma first attracted attention of such workers, most significant among these being those of Senft (1959); Threadgold (1963, a,b); Threadgold & Gallagher (1966); and Tay & Biagi (1968) on F. hepatica. Although no such study has so far been made on F. gigantica, there appears no possibility that at it's ultrastructural level would there be any specific deviation from the generalized pattern of F. hepatica.

The liver-flukes were generally believed to feed on the bile contents of liver and gall bladder and the much ramified caeca and multiple branched digestive system does provide sufficient testimony in this regard but based on the findings of Stephenson (1947b) and Gresson & Threadgold (1959), and his own, Dawes (1962,c & 1963) furnished conclusive evidence that F. hepatica feeds on the hepatic tissue of it's host as well.

Among other notable contributions on various trematode systems relating liver flukes are those of Lang (1880); Sommer (1880) and Bettendorf (1897) on it's generalized pattern

of nervous system. Bugge (1929); Kawana (1940) and Panteloures & Threadgold (1963) on the excretory system, and Shyamasundari & Rao (1975) on the neurosecretory cells of F. hepatica. These are substantiated by the histochemical studies of Weinland & Von Brand (1926); Home (1959b); Von Brand & Mercado (1961); Bjorkman et al., (1963); Pantelouris (1964b). Threadgold & Gallagher (1966) and Halton (1967 c,d). However, no such studies have been made on F. gigantica.

The reproductive system, particularly the Mehli's gland complex, cytology of gonads, spermatogenesis, oogenesis, germ cell cycle, and mechanism of egg shell formation have been subjected to elaborate studies by various investigators, Notable among these are the works of Gresson (1957); Yousufzai (1952, a,b; 1953a) and Rao (1959, a,b; 1960) on germ-cell cycles, spermatogenesis and oogenesis in F. hepatica; and of Yousufzai (1953b), and Smyth (1951) on egg-shell formation. However, the only study on this aspect in F. gigantica is that of Rao and Madhavi (1962) on Mehli's gland complex.

THE TEGUMENT

The generalised tegument of trematodes comprises an outer surface consisting of a triple layered plasma membarane extending over the surface of the spines. Next to the plasma membrane is the body of the tegument generally termed as 'matrix' followed by a basal layer. The basal layer is provided with a basement membrane which is followed by the outer circular and inner longitudinal (peripheral) muscles. The cytoplasmic matrix is in continuity with the tegumentary cells which lie in association with the parenchymal cells below the peripheral musculature.

Histological Observations:

The tegument of F. gigantica essentially comprises an outer matrix (Pl. I) which is about 20 μ thick and is delimited by a uniform strech of an ultra-thin membrane which may even be termed as epicuticle. The matrix is a uniform granular thick layer, extending into the acetabulum and the oral sucker where it is relatively thin. This layer is followed by a basal layer measuring about 5 μ , which is provided with a basement membrane to which are attached the peripheral muscles, the outer circular muscle layer and an inner longitudinal muscle layer. Circular layer measures about 10 μ while longitudinal layer about 15 μ thick.

No cell processes were observed to connect the spines.

Large specialized cells, termed as myoblasts, by Alvarado (1951) (Pl. I) are connected with the peripheral musculature. These are large flask-shaped cells, each about 30μ long, with large round central nucleus, about 8μ, and amoeboid nucleolus, about 3μ in size respectively.

Histochemical Observations:

The matrix is composed of certain glycoproteins or mucopolysaccharides showing distinctly positive (++) reaction with PAS, which is deastase resistant (Pl. IX, X). Some fat is also found in the matrix showing slightly positive (+) reaction with Sudan Black B. It is a highly protenacious layer showing strongly positive (+++) reaction with Mercurobromophenol blue. Upper part of the matrix contains acid muco-polysaccharide showing positive with Alcian blue.

Basal layer shows very characteristic staining property with Alcian blue similar to the connective tissue of vertebrates with Hydenhein's azan and Mallory's triple stain (Pl. XI2, XII 1). Some glycogen and fat is also found in it giving ++ive reaction with Best's Carmine, PAS, and Sudan Black B. The bound lipids are present in basal layer as a major component showing +++ive reaction with

Acetone Sudan Black (Pl. XII 2). The protein level is lower in this layer (+) with Mercurobromophenol blue.

Peripheral muscles possess glycogen showing ++ reaction with Best's Carmine and PAS (Pl. X 1,2) and a high quantity of protein showing +++ive reaction with Mercurobromophenol blue. It also shows an intense staining with Azocarmine G like nucleolus.

DISCUSSION

In an extensive study Alvarado (1951) concluded (a) a basal connective membrane, (b) a limiting or hyaline membrane, (c) a cuticular epithelium and (d) a true surface cuticle. Hyman (1951) reviewed the homologies and origin of the cuticle as follows: (a) that the 'Cuticle' is an altered and degenerated epidermis; (b) that it is the basement membrane of the former epidermis; (c) that it is the outer layer of an insunk epidermis, the cells and nuclei of which have sunk beneath the subcuticular musculature (d) that the cells in question are not epidermal but are parenchymal cells that secrete the 'Cuticle' and (e) that the 'Cuticle' is secreted by ordinary mesenchyme (parenchymal) not by special cells. The present studies are in accordance with the studies of Threadgold (1963). The cellular part of the tegument formerly termed as the sub-

cuticle consists of nucleated cells. The processes of these cells pass through the lacunae left by the network of peripheral musculature, which are clearly seen in frontal sections (Pl. II 1). This supports Threadgold's concept of cytoplasmic continuity between the two parts of the tegument. Evidently these cells are known to add secretory substances to the external part of the tegument which may take part in the nutritional uptake or resistance against host's substances as already postulated by Threadgold (1963) who has described the cuticle as a true cellular 'Tegument'. Further work in order to ascertain the functional significance of the structure is in progress. The matrix shows diastase resistant ++ive reaction with PAS, and similar condition was also observed by Berthier (1954); and Lal & Shrivastava (1960) in the tegument of F. hepatica for the presence of glyco - or muco-proteins. The upper most regions of the matrix appears to have acid mucopolysaccharide as tested with Alcian blue, and probably acts as antienzymatic substance against host's enzymes (Monné, 1959). The basal layer shows positive reaction with aniline blue like collagen which are also observed by Monné (1959) in F. hepatica and other trematodes but was not so clear.

Glycogen is absent in tegument which is confirmed by diastase digestion and Best's Carmine test, whereas

it's presence in F. hepatica tegument was mentioned by Pantelouris (1964a), though other workers like Von Brand & Mercado (1961) found a negative result for it in F. hepatica tegument. Some bound lipids are found in the basal layer which reveals the nature of this layer as a connective tissue.

THE PARENCHYMA

The parenchyma occupies major portion of platyhelminth interior. It comparises a network of loosely spaced connective tissue with large closely apposed cells appearing polygonal in sections. There also occur large vacoules which have been suggested as the spaces left due to the glycogen consumption (Pantelouris, 1965). The presence of mitochondria in these cells is indicative of active metabolic activities (Bjorkman and Thorsell, 1962). An electron dense intercellular layer, in between the parenchyma cells, much thicker than iterstitial material in other groups has been reported by Threadgold (1964).

The significant role of parenchyma in glycogen storage has already been suggested by Threadgold & Gallagher (1966).

Histological Observations:

As in other digenetic trematodes, the parenchyma is also a packing system comprising closely apposed cells in *F. gigantica*, greatly varying in shape and size from small round to somewhat elongated, measuring approximately 25-35 μ in cross section. The shape and size of the nuclei also vary considerably (Pl. I, II. Fig. 3), measuring from

5 to 6 μ , varying slightly in outline: being spherical, oval or sausage shaped. The nucleus usually lies towards one side of the cell. The cytoplasm of the parenchymal cells also vary greatly from a clear to highly granular form. Certain cells even possess large inclusion bodies of uncertain cytochemical nature. The intercellular space is thin at some places and markedly thick at other. The cells in the central parts which are not in close contact of other organ systems retain some what round shape while they tend to become elongated circumferencially around the intestinal caecae and excretory tubules. There are no direct contacts between the intestinal caeca and parenchymal cells, but there are very clear connective tissue lining in between. Similar lining though relatively thin also occurs around the reproductive organs. Parenchyma occupies space throughout the body around other organ systems. It is also in an indirect contact with the tegumentary system, which might probably assists in assimilation and storage of nutrients or other metabolites functions.

Histochemical Observations:

The parenchyma shows an intense reaction (+++) with Best's Carmine annd PAS (Pl. VIII, 2; IX, 2; and X, 2) which are non-resistant to diastase digestion (Pl. IX, 1). The result ascertains that glycogen is present in appreciable

quantity in the parenchymal cells and confirms that it is the principal glycogen storage system. Most intense reaction to glycogenophilic stains is shown by suckers and other muscular parts like the cirrus sac (Pl. IV, V, VI, VII, X and XI 1). Some bound lipid is also present in the parenchymal cells showing +ive colour to Acetone Sudan Black (Pl. XIII). These might be glycolipids, phospholipids or lipoproteins. The protein level is found slightly lower (+) with Mercurobromophenol blue. The histochemical results are tabulated in Table II.

DISCUSSION

The parenchyma is essentially similar to that of F. hepatica. It is composed of closely apposed cells of various size and shape. The intercellular materials greatly vary in thickness and appearance, which may be regarded as the interstitial material as described by Threadgold & Gallagher (1966) for F. hepatica. The system is in contact with other organ systems like intestinal caeca , excretory system, reproductive system, and tegumentary system which reveals that all the exchanges of materials in physiological (metabolic) activities, take place through the parenchyma. According to this view the parenchyma must be regarded as the site of principal metabolic activities,

and it compensates the absence of circulatory system.

Glycogen storage is performed by the parenchyma, as has been reported for *F. hepatica* by Von Brand & Mercado (1961). There are present glycogen reserves of all sizes.

Presence of fat is mostly in the form of bound lipids, and this fat moiety is derived probably through the sterols present in the host blood, and conforming the fat that it also feeds on blood as suggested by Ben Dawes (1963a,b,c) in *F. hepatica*.

TABLE I. INCIDENCE OF FASCIOLA GIGANTICA AT ALIGARH. (Based on sample survey)

Month/Year	Total Hosts Examined	Positive Hosts	% age of infection	Maximum Worm Burden
April, 1974	787	12	1.52	50
May	988	40	4.04	200
June	831	3	0.36	100
July	102	6	5.88	29
Aug.	96	3	3.16	30
Sept.	121	5	4.13	81
Oct.	117	5	4.27	48
Nov.	99	3	3.03	27
Dec.	122	7	5.74	44
Jan., 1975	100	2	2	1700
Feb.	82	2	2.44	30
March	122	2	1.64	14
April	110	2	1.82	23
May	106	1	0.94	4
June	77	Nil	Nil	Nil
July	89	1	1.12	17
Aug.	95	1	1.05	23
Sept.	101	4	3.96	77
Oct.	74	3	4.06	29
Nov.	117	6	5.16	48
Dec.	124	3	2.42	40
Jan., 1976	83	6	7.23	27
Feb.	113	2	1.77	38

TABLE II. Results of various histochemical tests performed on the tegument and parenchyma of *Fasciola gigantica*

S.No.	Substance investigated	Histochemical test performed	Tegument				Parenchyma	Remarks
			spine	matrix	basal layer	peripheral muscles		
1.	Glycogen	Best's Carmine	-	-	++	++	+++	
2	Glycogen and other polysaccharides	PAS	-	++	++	++	+++	Matrix is diastase resistan
3	Lipids	Sudan Black B	-	+	++	-	-	
4	Bound Lipids	Acetone-Sudan Black	-	-	+++	-	+	
5	Protein	Mercuro bromophenol blue	++	+++	+	+++	+	
6	Acid mucopolysaccharide	Alcian blue	-	+	-	-	-	Only upper region of the matrix shows (+) reaction, but not the whole matrix

Reaction index:

Intensely stained: +++, Slightly stained: +

Moderately stained: ++, Negative: -

EMPIRICAL STUDIES

Metabolic studies on helminth parasites were heralded by Weinland (1901, 1904). Since then many investigators have richly contributed to such aspects and notable contributions in this regards are those of Lueding (1949); Von Brand (1952, 72); Smyth (1966) and Erasmus (1972) has extensively reviewed the subject a numerous investigations have since followed. As compared to cestodes less work has been done on trematodes. Mostly such studies are on Fasciola hepatica. Certain concise studies on glycogen protein and lipid metabolism of F. gigentica has been made by Goil (1958a,b; 1961). In the present study the total glycogen and fat has been estimated and compared with the figures obtained by Goil (1958, 1961).

Total Glycogen:

Glycogen was extracted from the tissue according to the method of Ashman and Seed (1973) and determined spectrophotometrically by the method of Montgomerry (1957). The result of twenty analyses show glycogen content of F. gigantica ranging from 4.9 to 5.35% with an average of 5.134 ± 0.034% of fresh tissue weight (Table III).

Total Fat:

Fat was extracted by the Kjehldahl's soxhlet method

through petroleum ether. The result of ten analyses indicate that the fat content of F. gigantica ranged from 2.74 to 3.58% with an average of 3.2148 (±0.102)% of fresh tissue weight.

DISCUSSION

The glycogen is present in significant amount in F. gigantica. Histochemically it has been accertained that the glycogen is mostly present in parenchyma and muscular parts of the F. gigantica. The figures confirm the same moiety found by Goil (1961), whose figure was slightly higher (5.4%), while El-hehyawi estimated 4.3% total glycogen content. It also shows that this figure lies with in the range given by Von Brand (1972) for F. hepatica.

The presence of fat in F. gigantica supports the histochemical localization of the same. The least worked out aspect is the metabolism of fat in helminthes. The fat most probably is derived from the sterols of host blood. Present study shows that fat content of F. gigantica ranges from 2.74 to 3.58% with an average of 3.2148 (±0.102)% of fresh tissue weight, while Goil (1958) found it as 2.81 (± 0.14)% of f.w. for the same worm, which are slightly lower than present figures, but however, it does not vary much and still supports Goil's (1958) finding.

Although these figures are slightly higher than those obtained by Goil (1958) yet they approximately fall within the same range, particularly when Goil (opcit) has indicated that lipid content q these fluke tend to increase during or, as a result of starvation: He had obtained similar data through subjecting her life samples to starvation conditions as well.

TABLE IV. Photometric estimation of glycogen in fresh tissue of *Fasciola gigantica*

No. of obs.	gm fat/2.5 gm each fresh tissue	gm fat/100 gms fresh tissue 'x'	difference of mean an 'x' (d)	d^2
1	0.0873	3.492	0.0772	0.06682984
2	0.0860	3.440	0.2252	0.05071504
3	0.0798	3.192	0.056	0.003136
4	0.0802	3.208	0.0068	0.00004624
5	0.0766	3.064	0.1508	0.02264064
6	0.0895	3.580	0.3652	0.13337104
7	0.0779	3.116	0.0988	0.00976144
8	0.0699	2.769	0.5812	0.33779344
9	0.0880	3.52	0.3052	0.09314704
10	0.0685	2.740	0.4748	0.22343504
		$x = 32.148$		$\Sigma d^2 = 0.94087576$

$$\text{Mean } x = 3.2148$$

$$\text{S.D.} = \frac{0.94087576}{9} = 0.32332$$

$$\text{S.E.} = \frac{0.32332}{10} = 0.102$$

The result of ten analyses show that fat content of *Fasciola gigantica* range from 2.74 to 3.58% with an average of 3.2148 ± 0.102 of fresh tissue weight.

TABLE III. Photometric estimation of glycogen in fresh tissue of *Fasciola gigantica*

No. of obns.	% age Transmittance	O.D.	Formula $\frac{O.D. \times 8500}{1000}$ = 'x' glycogen/100 gms. fresh tissue	Difference of mean and 'x' 'd'	d^2
1	25	0.602	5.117	0.017	0.000289
2	25	0.602	5.117	0.017	0.000289
3	23.5	0.629	5.3465	0.2125	0.04515625
4	26.5	0.577	4.9045	0.1295	0.01678025
5	26.0	0.585	4.9725	0.161	0.025921
6	26	0.585	4.9725	0.161	0.025921
7	23	0.638	5.423	0.289	0.025921
8	25.5	0.594	5.049	0.085	0.007225
9	27	0.569	4.8365	0.2975	0.08850625
10	26.5	0.577	4.9045	0.1295	0.01678025
11	23	0.638	5.423	0.289	0.025921
12	25	0.602	5.117	0.017	0.000289
13	24.5	0.611	5.1935	0.0595	0.00354025
14	23	0.638	5.423	0.289	0.025921
15	25	0.602	5.117	0.017	0.000289
16	26	0.585	4.9725	0.161	0.025921
17	23.5	0.629	5.3465	0.2125	0.04515625
18	25.5	0.594	5.049	0.085	0.007225
19	25.5	0.594	5.049	0.085	0.007225
20	23.5	0.629	5.3465	0.2125	0.04515625

$X = 102.68$ $\quad \Sigma d^2 = 0.43943275$

$$\text{Mean } x = 5.134$$

$$\text{S.D.} = \frac{d}{N-1} = \frac{0.43943275}{19} = 0.15208$$

$$\text{S.E.} = \frac{\text{S.D.}}{N} = \frac{0.15208}{4.472} = \pm 0.034$$

Glycogen content ranges from 4.9 to 5.35% with an average of 5.134 ± 0.034 of fresh tissue weight.

CONCLUSIONS

(1) The average incidence of *F. gigantica* at Aligarh is about 3% in buffaloes, which is increasing gradually year by year. Subsequent increase in incidence may accrue greater economic losses to cattle industry.

(2) The tegument of *F. gigantica* is essentially similar to that of *F. hepatica*. There are two types of cells which comunicate through fine processes. These processes pass across the network of peripheral musculature; and these can be observed conveniently in frontal sections.

Spines are projected anteroposteriorly in *to to*.

The basal layer reflects a nature of connective tissue as widened by with staining Aniline blue staining reaction. This layer provides a rest for the spinal base.

The matrix is glycoprotein or mucoprotein in chemical nature whose upper region is composed of acid mucopolysaccharide. which is an antienzymatic substance. The basal layer possesses some bound lipid.

(3) The parenchymal cells vary in their shape at different places. Nuclei of parenchymal cells are of polytypic. They are accentrically situated. The parenchymal cells appear elongated near tegumentary system and become elongated

circumferencially around the intestinal caeca and excretory tubules.

The parenchyma has been found to be the principal site of glycogen storage and is essentially associated with glycogenosis. Bound lipids, (presumably phospholipids) have also been histochemically found in this system.

(4) Quantitatively, glycogen has been found ranging from 4.9 to 5.35% with an average of 5.134 (± 0.034)% of fresh tissue weight which is slightly lower than the figures given by Goil (1961) for the same moiety which is 5.4% (±0.16)% of fresh weight; it also lies with in the range given by Von Brand (1972) for F. hepatica. The fat content, as a result of ten analyses, is found ranging from 2.74 to 3.58% with an average of 3.2148 (± 0.102)% of fresh tissue weight, which is slightly higher than the fat moiety found by Goil (1958) in the same worm which was 2.81 (±0.14)% of fresh weigh.

REFERENCES

Alvarado, R. (1951a).

El tegumento, la musculature t el parenquima de fasciola hepatica. Trab. Inst. Cienc. nat. Acosta. 3(1): 1-90.

Ashman, P.U. & Seed, J.R. (1973).

Biochemical studies on the role, Microtus montanus - 1. The daily variation of hepatic glucose-6-phosphatase and liver glycogen. Comp. Biochem. Physiol., 45B(2): 365-378.

Berthier, L. (1954).

Contribution a l' etude du tegument de de Fasciola hepatica L. Arch. Zool. exp. gen., 91: 89-102.

Bettendorf, H. (1897).

Uber Muskulature und Sinneszellen der Trematoden. Zool. Jahrb. abt. Anat., 10(3): 307-358.

Bhalerao, G.D. (1935c).

Helminth. parasites of the domesticated animals in India. Sci. Monogr. Goun. Agric. Res. India, No. 6, 365 pp.

Bjorkman, N. & Thorsell, W. (1962).

The fine morphology of the mitochondria from the parenchymal cells in the liver fluke (Fasciola hepatica L.). Exper. Cell Res., 27(2): 342-346.

Bjorkman, N., Thorsell, W. & Lienert, E. (1963).
Studies on the action of some enzymes on the cuticle of Fasciola hepatica L. Experientia, 19(1): 3-5.

Bueding, E. (1949).
Metabolism of parasitic helminths. Physiol. Rev., 29: 195-218.

*Clegg, J.A. (1957).
Studies on the maintenance of Fasciola hepatica in vitro Ph.D. thesis, University of London.

Cobbold, T.S. (1855).
Description of a new trematode (Fasciola gigantica). Edinb. N. Phil. J. n.s., 2(2): 262-266.

Dawes, B. (1954a).
Maintenance in vitro of Fasciola hepatica. Nature, 174: 654-655.

Dawes, B. (1962c).
A histological study of the caecal epithelium of Fasciola hepatica L. Parasitology, 52(3/4): 483-493.

Dawes, B. (1963a).
The migration of juvenile forms of Fasciola hepatica L. through the wall of the intestines in the mouse, with some observations on food and feeding. Parasitology, 53(1,2): 109-122.

Dawes, B. (1963b).

Some observations of Fasciola hepatica L. during feeding operations, in the hepatic parenchyma of the mouse, with notes on the nature of liver damage in the host. Parasitology, 53(1,2): 135-144.

Dawes, B. (1963c).

Hyperplasia of the bile duct in fascioliasis and it's relation to the problem of nutrition in the liver-fluke. Parasitology, 53(1,2): 123-134.

El-Hehyawi, G.H. (1969).

Biochemical studies on trematodes 1. lipid fractions and glycogen content of Fasciola gigantica. J. Vet. Sc. U.A.R. Cairo, 6(2): 159-170.

Erasmus, D.A. (1972).

The Biology of Trematodes: Edward Arnold, University Press: Belfast. pp. 312.

Goil, M.M. (1958a).

Fat metabolism in trematode parasites. Z. Parasitkde., 18(4): 320-323.

Goil, M.M. (1958b).

Protein metabolism in trematode parasites. J. Helminth., 32(3): 119-124.

Goil, M.M. (1961).

Physiological studies on trematodes - Fasciola gigantica carbohydrate metabolism. Parasitology, 51(3/4): 335-337.

Gresson, R.A.R. (1957).

Spermatoleosis in Fasciola hepatica. Quart. J. micr. Sci., 98(4): 493-498.

Gresson, R.A.R. & Threadgold, L.T. (1959a).

A light and electron microscope study of the epithelial cells of the gut of Fasciola hepatica L. J. biophys. biochem. Cytol., 6(2): 157-162.

Halton, D.W. (1967c).

Histochemical estirase of carboxylic estirase activity in Fasciola hepatica. J. Parasit., 53(6): 1210-1216.

Halton, D.W. (1967d).

Studies on glycogen deposition in trematodes. Comp. Biochem. Physiol., 23(1): 113-120.

Hyman, L.H. (1951).

The invertebrates: Platyhelminthes and Rhynchocoela. The acoelomate Bilateria. Vol. II. McGraw-Hill New York. pp. 550.

Jackson, H.G. (1921).

A revision of the genus Fasciola, with particular reference to F. gigantica (Cobbold) and F. nyanzi (Leiper). Parasitology, 13(1): 48-56.

Kawana, H. (1940).

Study on the development of the excretory system of Fasciola hepatica L. with special reference to it's

first intermediate host in Central China. J. Shinghai Sci. Inst., 5(1): 13-34.

Kendall, S.B. (1954a).

Fascioliasis in Pakistan. Ann. trop. Med. Parasit., 48(3): 307-313.

Kibakin, V.V. (1961).

The epizootology of fascioliasis in sheep in the Turkmen. S.S.R. (Russian text). Conference (IV) on Problems in Parasitology, Alma Ata, (1959): 389-393.

Lal, M.B. & Shrivastava, S.C. (1960).

Some histochemical observations on the cuticle of Fasciola indica Varma 1953. Experientia, Basle, 16: 185-186.

*Lang, A. (1880).

Untersuchungen zur vergleichenden Anatomie und histologie des Nervensystems der Plathelminthen. II. Uber das Nervensystem der Trematoden. Mitt. zool. Sta. Neapel, 2(1): 28-52.

*Leuckart, R. (1881c).

Zur Entwicklungsgeschichte des Leberegels. Zool. Anz., (99) Vol. 4: 641-646.

Leuckart, R. (1886a).

Die Parasiten des Menschen und die von ihnen herrührenden Krankheiten. Ein Hand-und Lehrbuch fur

Naturforscher und Aerzte. 2. Aufl., V. 1,3: 855-1000 pp. Leipzig. u. Heidelberg.

Lillie, R.D. (1965).
Histopathologic Technic and Practical Histochemistry. 3rd (ed.). McGraw-Hill New York. pp. 715.

Lutz, P.L. & Siddiqi, A.H. (1962).
Composition of haemoglobin of Fasciola gigantica (Trematoda:Digenea) and it's host. Expl. Parasit., 20(1): 83-87.

MacCoy, O.R. (1935).
The physiology of the helminth parasites. Physiol. Rev., 15(2): 221-240.

Monné, L. (1959b).
On the external cuticles of various helminths and their role in the host-parasite relationship. Ark. Zool. Stockholm, 12(4): 343-358.

Montgomery, R. (1957).
Determination of glycogen. Arch. Biochem. Biophys., 67: 378-386.

Muller, W. (1923).
Die Nahrung von Fasciola hepatica und ihre Verdauung. Zool. Anz., 57(9-13): 273-281.

Pantelouris, E.M. (1964b).

Localization of glycogen in Fasciola hepatica L. and an effect of insulin. J. Helminth., 38(3/4): 283-286.

Pantelouris, E.M. (1965).

The Common Liver Fluke. Pergamon Press, London, 259 pp.

Pantelouris, E.M. & Threadgold, L.T. (1963).

The excretory system of the adult Fasciola hepatica L. La Cellule, 64(1): 63-67.

Patnaik, M.M. (1971).

Studies on geographical distribution and ecology of Fasciola gigantica with special reference to it's intermediate host. Lymnaea auricularia var. rufescens in Orissa. Indian. J. Helminth., 23(2): 115-134.

Pearse, A.G.E. (1960).

Histochemistry: Theoretical and Applied. 2nd (ed.), J & A. Churchill. 998 pp.

Prenant, M. (1922).

Recherches sur la parenchyme des helminthes, essai d'histologie comparee. Arch. Morphol., 5(1): 1-474.

Rao, K.H. (1959a).

Observations on the Mehli's gland complex in the liver fluke, Fasciola hepatica. J. Parasit., 45(3): 347-351.

Rao, K.H. (1959d).

Histochemistry of Mehli's gland and egg-shell formation in the liver fluke. Experientia, 15(2): 464.

Rao, K.H. (1960).

The problem of Mehli's gland. Parasitology, 50(3/4): 349-350.

Rao, K.H. & Madhavi, R. (1962).

Mehlis's gland complex in the cattle liver fluke Fasciola gigantica Cobbold. Curr. Sc. Bangalore, 31(3): 101-102.

Roy, S.C. (1954).

Liver fluke disease amongst cattle in the district of Darjeeling with it's main source of infection. Proc. Indian. Sc. Congr., 41st (1954), Part III: 224-225.

Sarwar, M.M. (1957).

Fasciola indica Varma, a synonym of F. gigantica Cobbold. Biologia, Lahore, 3(2): 168-175.

Senft, A.W. (1959b).

Electron microscope study of the integument, flame cells and gut of the human parasite, Schistosoma mansoni. Biol. Bull. WoodsHole, 117(2): 367.

Shyamsundri, K. & Rao, K.H. (1975).

The structure and cytochemistry of the neurosecretory cells of Fasciola hepatica L. Z. Parasitenk., 47(2): 103-109.

Siddiqui, A.H. & Lutz, P.L. (1966).

Osmotic and ionic regulation in Fasciola gigantica (Trematoda:Digenea). Expl. Parasit., 19 (3): 348-357.

Smyth, J.D. (1951).

Egg-shell formation in Trematodes and Cestodes as demonstrated by the methyl or malachite green techniques. Nature, (Lond.), 168: 322-323.

Smyth, J.D. (1966).

The Physiology of Trematodes. Oliver & Boyde, London, pp. 256.

Sommer, F. (1880).

Die Anatomie des Leberegels Distomum hepaticum L. Z. wiss. Zool., 34(4): 539-640.

Stephenson, W. (1947a).

Physiological and histochemical observations on the adult liver fluke, Fasciola hepatica L. Survival in vitro. Parasitology, 38(3): 116-122.

Stephenson, W. (1949b).

Physiological and histochemical observations on the adult liver fluke, Fasciola hepatica L. II. Feeding. Parasitology, 38(3): 123-127.

Stephenson, W. (1947c).

Physiological and histochemical observations on the adult liver fluke, Fasciola hepatica L. III. Egg-shell formation. Parasitology, 38(3): 128-139.

Stephenson, W. (1947d).

Physiological and histochemical observations on the adult liver fluke, Fasciola hepatica L. IV. The excretory system. Parasitology, 38(3): 140-144.

*Stossich, M. (1892).

I distomi degli uccelli. Lavoromonografico. Boll. Soc. Adriat. Sc. Nat. Triete., 13(2): 143-196.

Tay, J. & Biagi, F.F. (1968).

Ultrastructura de F. hepatica. I. cuticula, glandulas vitelogenas, Ciegos intestinales y Celulas en flama. La prensa med. Mex., 33(11-12): 397-405.

Thapar, G.S. & Tandon, R.S. (1952).

On the life history of liver-fluke, Fasciola gigantica Cobbold, 1855, in India. Ind. J. Helm., 4(2): 1-36.

*Thomas, A.P., (1881).

Reports of experiments on the development of the liver-fluke (Fasciola hepatica). J. Roy. Agric. Soc. England, 17(1): 1-29.

*Thomas, A.P., (1882a).

The rot in sheep, or the life history of the liver-fluke. Nature, 26: 606-608.

Thomas, A.P. (1883a).

The life history of the liver fluke. Quart. J. Micr. Sci., 23(1): 99-133.

*Thomas, A.P. (1883b).

The natural history of the liver fluke and the prevention of rot. J. Roy. Agric. Soc. (England). 19(4): 276-305.

Threadgold, L.T. (1963a).

The ultrastructure of the "cuticle" of Fasciola hepatica. Exptl. Cell Res., 30(1): 238-242.

Threadgold, L.T. (1963b).

The tegument and associated structures of Fasciola hepatica. Quart. J. Micr. Sci., 104(4): 505-512

Threadgold, L.T., (1964).

Electron microscope studies of Fasciola hepatica, VI. The ultrastructural localization of phosphatases. Expl. Parasit., 23(2): 264-276.

Threadgold, L.T. & Gallagher, S.S.E. (1966).

Electron microscope studies of Fasciola hepatica. 1. The ultrastructure and interrelationship of the parenchymal cells. Parasitology, 56(2): 299-304.

Varma, A.K. (1953).

On Fasciola indica n.sp. with some observations on F. hepatica and F. gigantica. J. Helminth., 23 (3/4): 185-198.

Von Brand, T. (1952).

Chemical Physiology of Endoparasitic Animals. Academic Press, New York. 339 pp.

Von Brand, T. (1972).

Biochemistry of Parasites. 2nd (ed.). Academic Press, New York. pp.

Von Brand, T. & Mercado, T. I. (1961).

Histochemical glycogen studies on Fasciola hepatica. J. Parasit., 47(3): 459-463.

Watanabe, S. (1958).

General review on Fascioliasis in Japan. J. Jap. vet. med. Ass., 11: 293-299.

Watanabe, S. & Iwata, S. (1956c).

The morphological studies on the genus Fasciola in Japan. II.(Proc. 41. Med. Japan. Soc. vet. Sc., Tokyo, Apr. 5-6). Japan. J. Vet. Sc., Tokyo, Vol. 8, Suppl Dec. (Japanese text), pp. 62-63.

Watanabe, S. & Iwata, S., (1956a).

Morphological studies of the genus Fasciola in Japan, with particular reference to F. gigantica Cobbold. (Proc. 27. Ann. Meet. Japan Soc. Parasitol.) (Japanese text) Kiseichumaku Zasshi, 7(6): 231.

Watanabe, S. & Ueno, H. (1959a).

Morphological studies of the genus Fasciola in Japan, with particular reference to F. hepatica and F. gigantica. (Proc. 28. Ann. Meet. Japan. Soc. Parasitol., Apr. 6-7) Kiseichumaku Zasshi, 8(3): 368-369.

Watanabe, S. & Ueno, H. (1960a).

A revision of the genus Fasciola, with particular reference to Japanese species. Kiseichugaku zasshi, 9(4): 379.

Watanabe, S. & Ueno, H. (1960b).

Morpholigical studies on the genus Fasciola with, particular reference to Japanese species. Japan. J. vet. Sc. Tokyo, 22: 472.

*Weinland, E. (1901).

Uber kohlehydratzersetzung ohne Sauerstoffaufnahme bei Ascaris, einen tierischen Garungsprozess. Ztschr. Biol., 42:55-90.

*Weinland, E. (1904).

Uber die Zersetzung Stickstoffhaltiger Substanz bei Ascaris. Ztschr. Biol. 45:517-531.

*Weinland, E. & Von Brand, T. (1926).

Beobachtungen au Fasciola hepatica. Z. vergl. Physiol. 4(2): 212-285.

Yousufzai, H.K. (1952a).

Cytological studies on the spermatogenesis of Fasciola hepatica L. Cellule, 55(1): 5-19.

Yousufzai, H.K. (1952b).

Female reproductive system and egg-shell formation in Fasciola hepatica L. Nature. 169: 549.

Yousufzai, H.K. (1953a).

Cytological studies on the oogenesis of Fasciola hepatica.

L. Cellule, 55(1): 23-42.

Yousufzai, H.K.(1953b).

Shell gland and egg-shell formation in Fasciola hepatica

L. Parasitology, 43(1): 88-93.

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* References not seen in original.

LEGEND

Abbreviations:

bl	=	basal layer
cm	=	circular muscle
cp	=	cell process
et	=	excretory tubules
ma	=	matrix
mb	=	myoblast
n	=	nucleus
nl	=	nucleolus
pc	=	parenchymal cell
sp	=	spine
tc	=	tegumentary cell

PLATE I. Camera lucida diagram showing tegument and parenchyma of *Fasciola gigantica* in T.S.

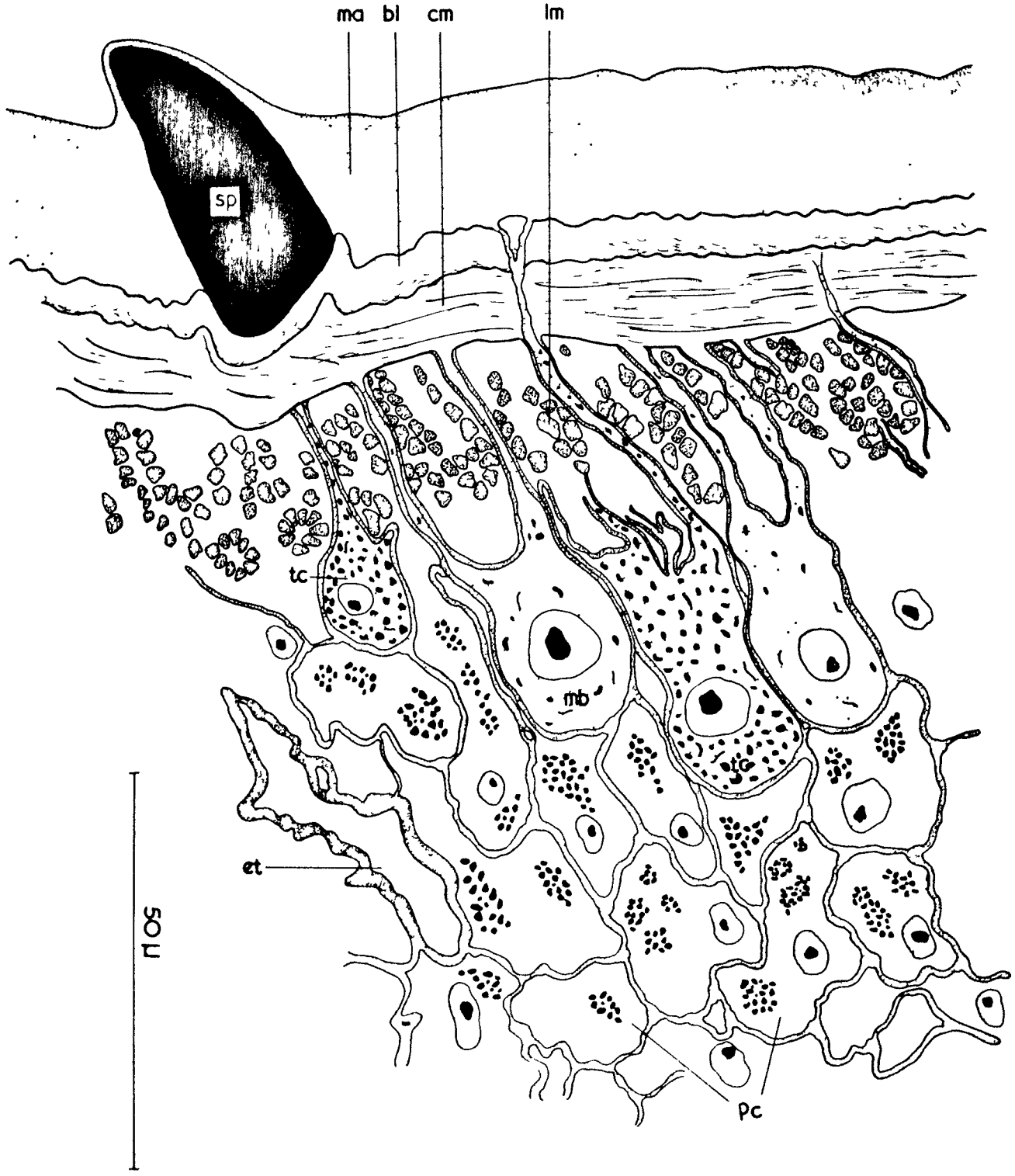


PLATE I

PLATE II. 1. Camera lucida diagram showing peripheral muscular ag;rmagment in frontal section.

2. Different types of nuclei of parenchymal cells.

3. Diagram showing disposition of the spines in <u>to</u> <u>to</u>

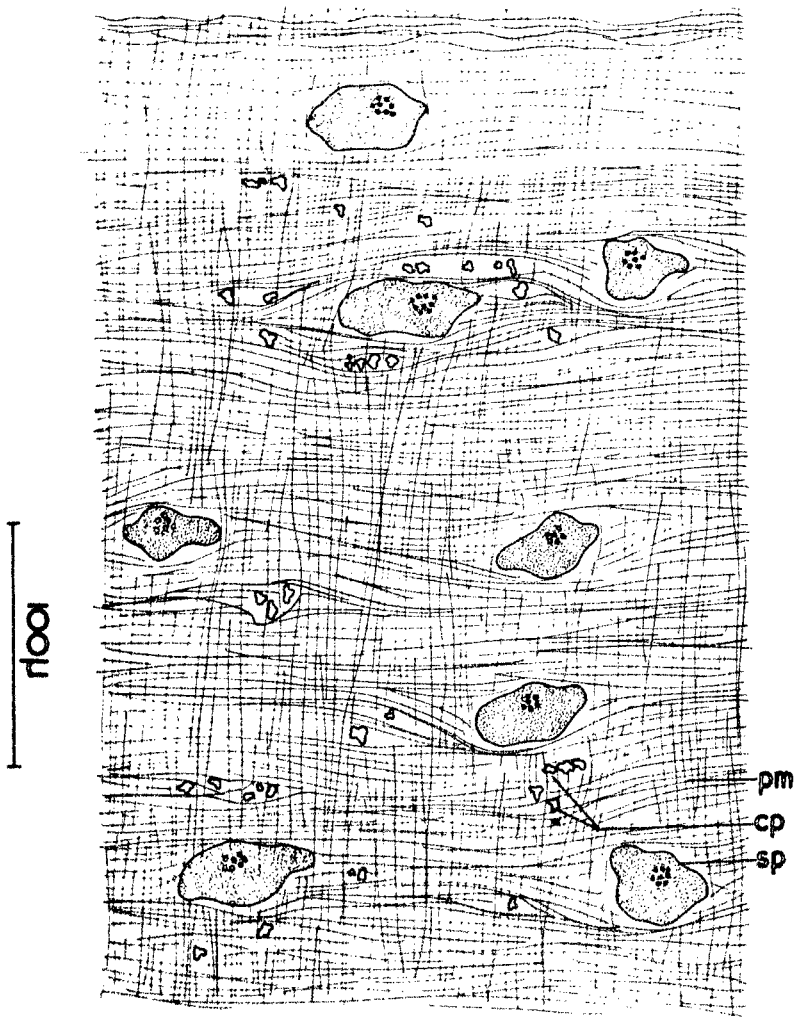


Fig. 1

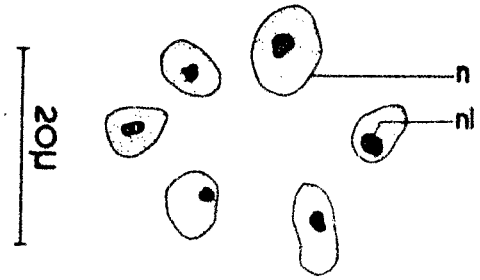


Fig. 2

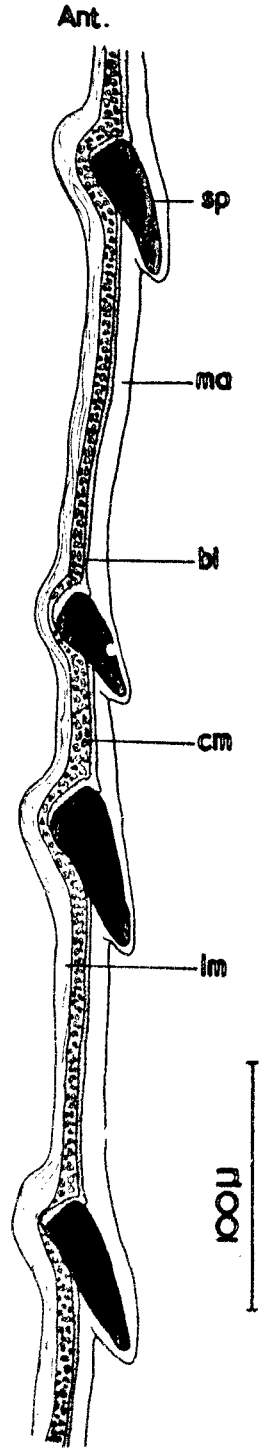


Fig. 3

PLATE II

PLATE III.

Standard curve showing the quantitative glycogen through 'Spectronic 20'

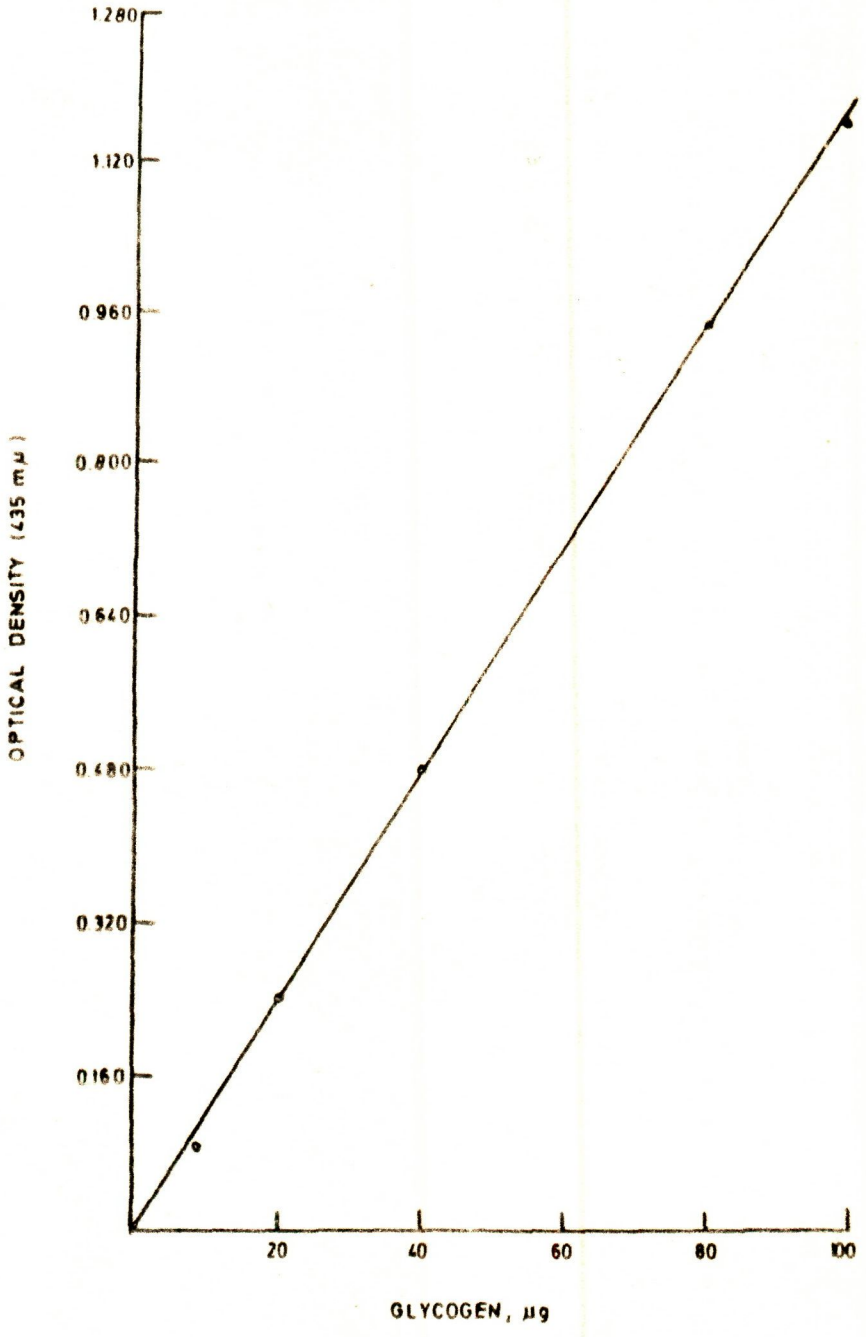

1.280
1.120
0.960
0.800
0.640
0.480
0.320
0.160
OPTICAL DENSITY (435 mμ)
20
40
60
80
100
GLYCOGEN, μg

PLATE III

PLATE IV. 1. T.S. through oral sucker of *Fasciola gigantica*-Best's Carmine.

2. T.S. through oral sucker of *F. gigantica*-PAS.

(1)

(2)

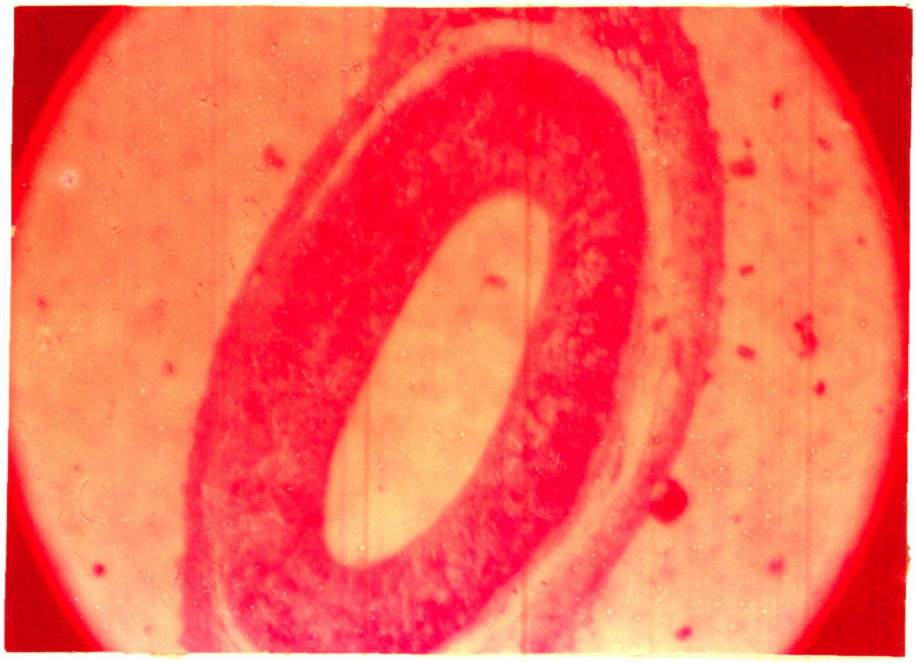
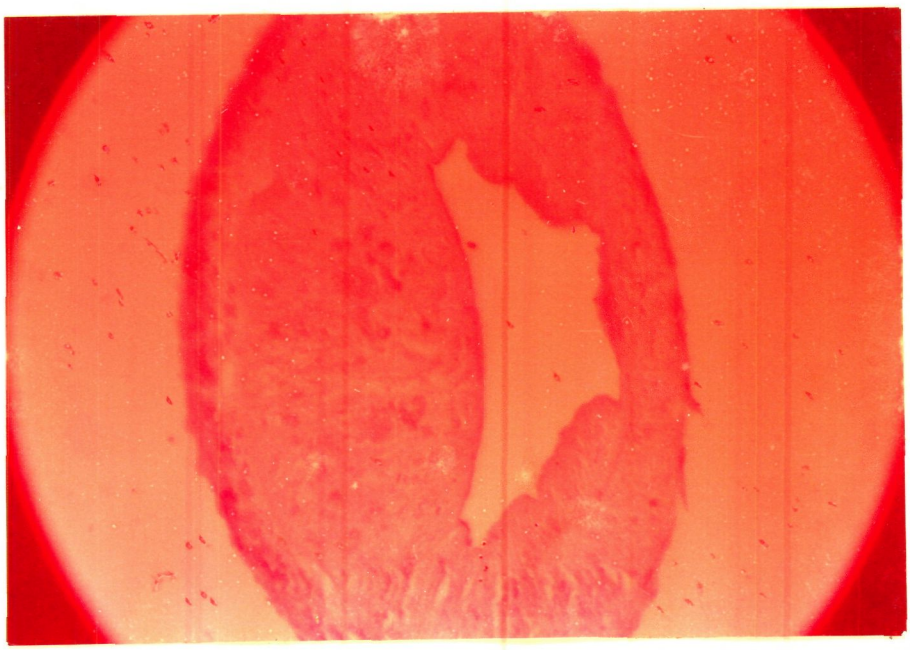


PLATE IV

PLATE V. 1. T.S. through oral sucker of *F. gigantica* — Best's Carmine (Predigested with ∝- amylase).

2. T.S. through oral sucker of *F. gigantica* — PAS (Predigested with ∝- amylase).

(1)



(2)

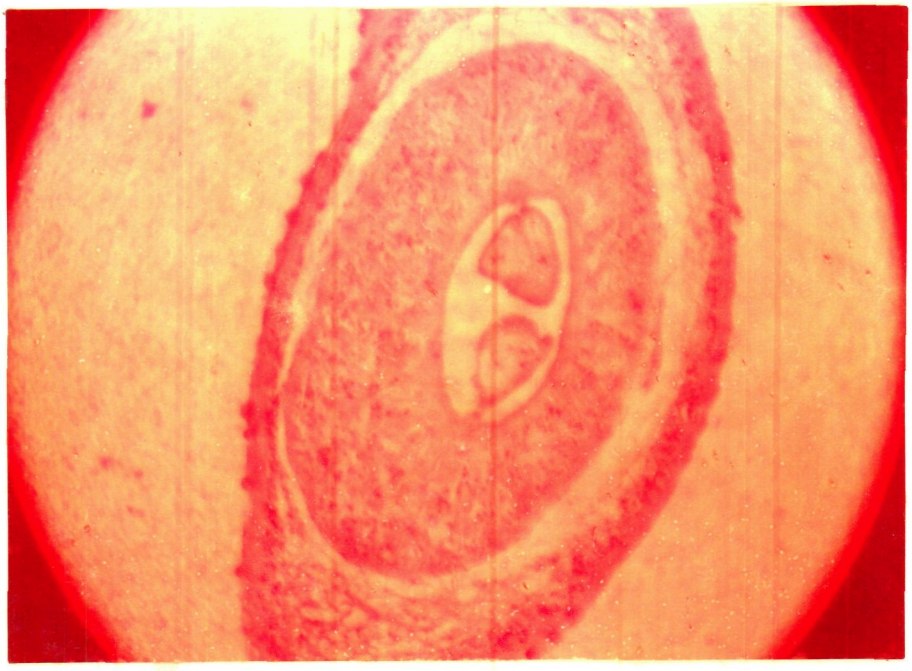
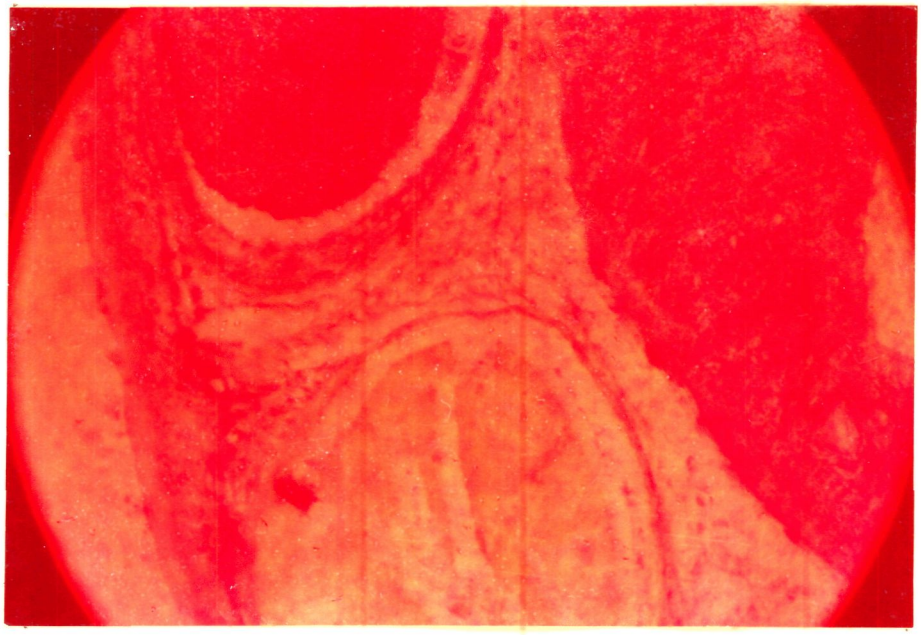


PLATE V

PLATE VI. 1. T.S. of *F. gigantica* through V.S. and cirrus sac — Best's Carmine

2. T.S. of *F. gigantic* through V.S. and cirrus sac — PAS.

(1)



(2)

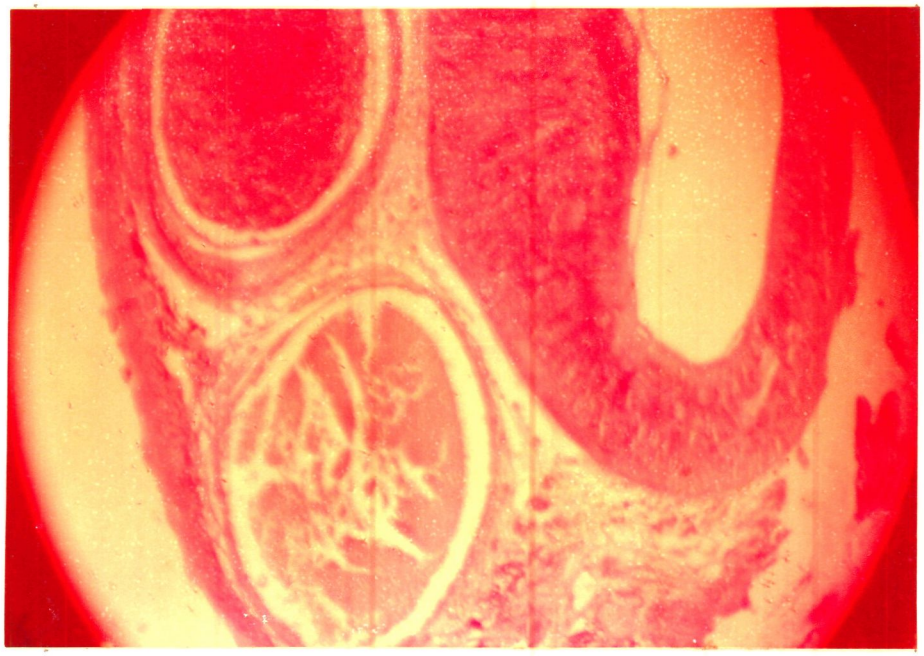
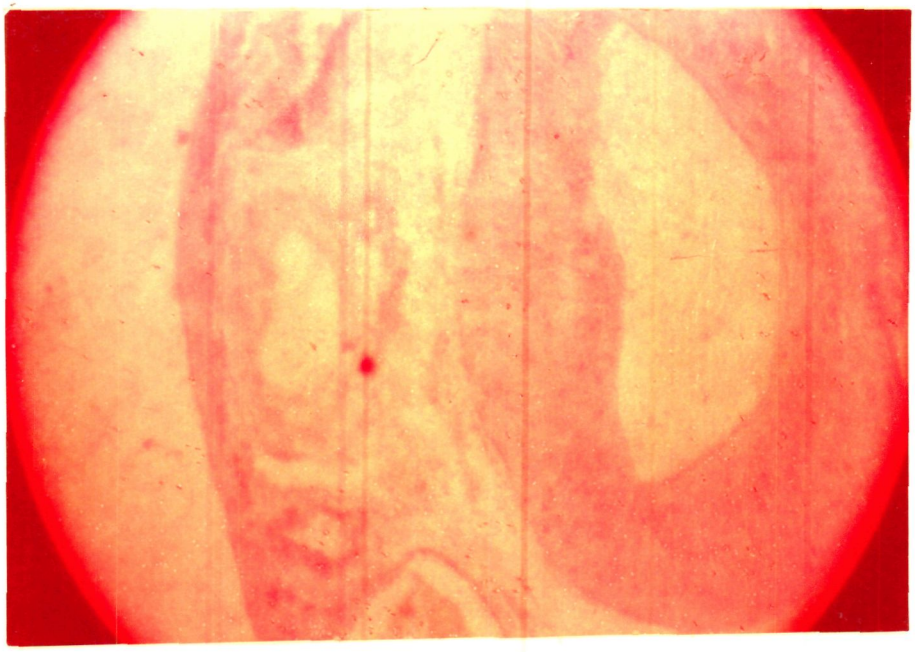


PLATE VI

PLATE. VII. 1. T.S. of *F. gigantica* through V.S. and cirrus sac — Best's Carmine (Predigested with α - amylase).

2. T.S. of *F. gigantica* through V.S. and cirrus sac — PAS (Predigested with α -amylase).

(1)



(2)

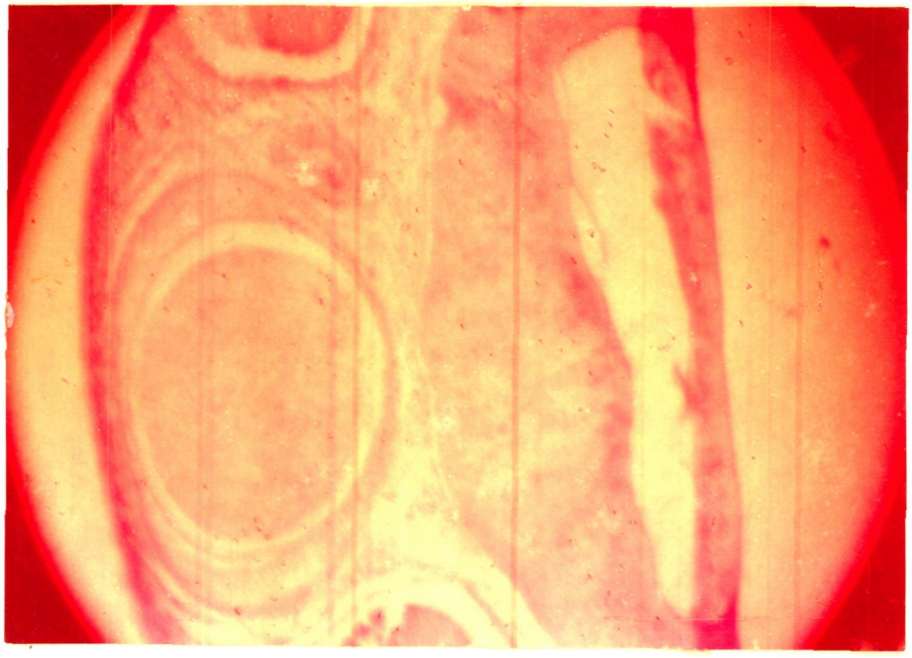
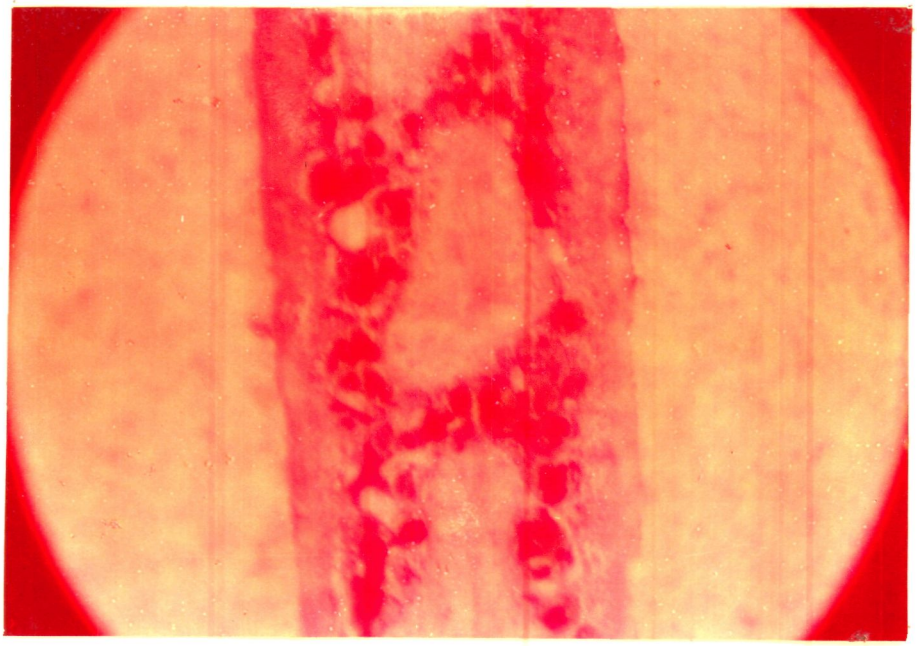


PLATE VII

PLATE. VIII. 1. T.S. of F. gigantica through middle parenchyma — Best's Carmine.

2. T.S. of F. gigantica through middle parenchyma — PAS.

(1)



(2)

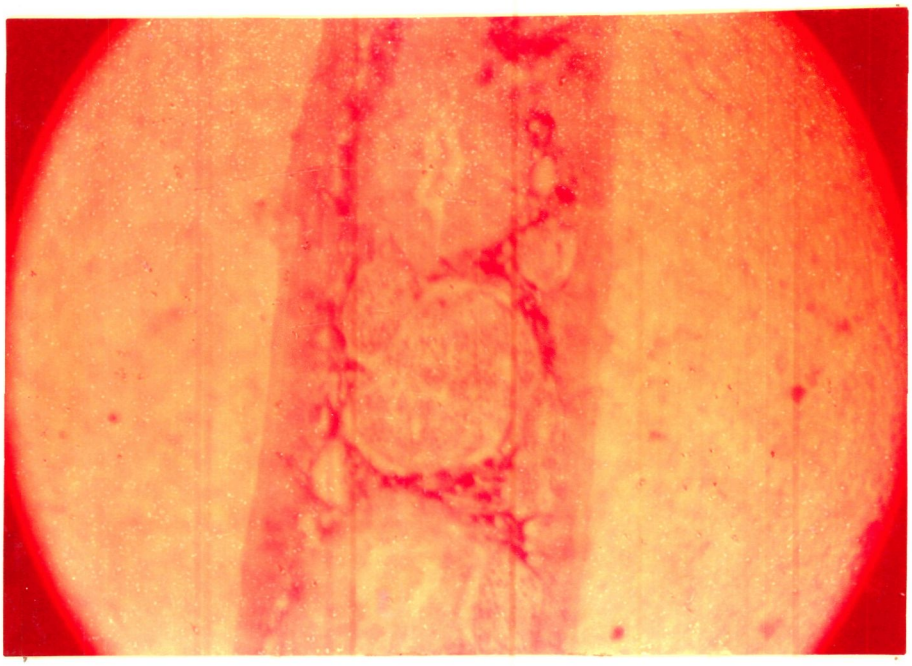
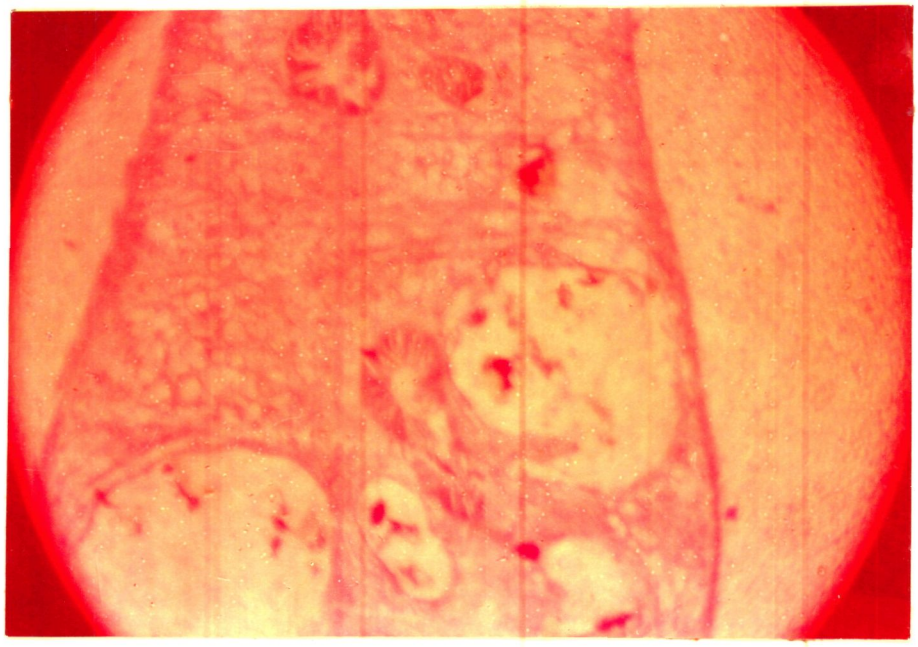


PLATE VIII

TABLE. IX. 1. T.S. of *F. gigantica* through middle parenchyma — Best's Carmine (Predigested with α - amylase).

2. T.S. of *F. gigantica* through middle parenchyma — PAS (Predigested with α - amylase).

(1)



(2)

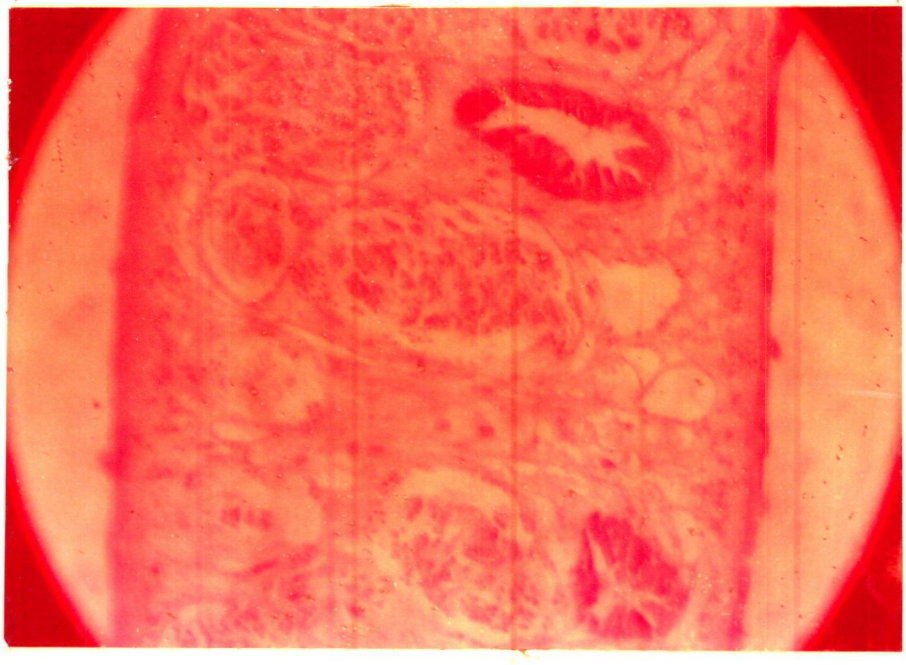
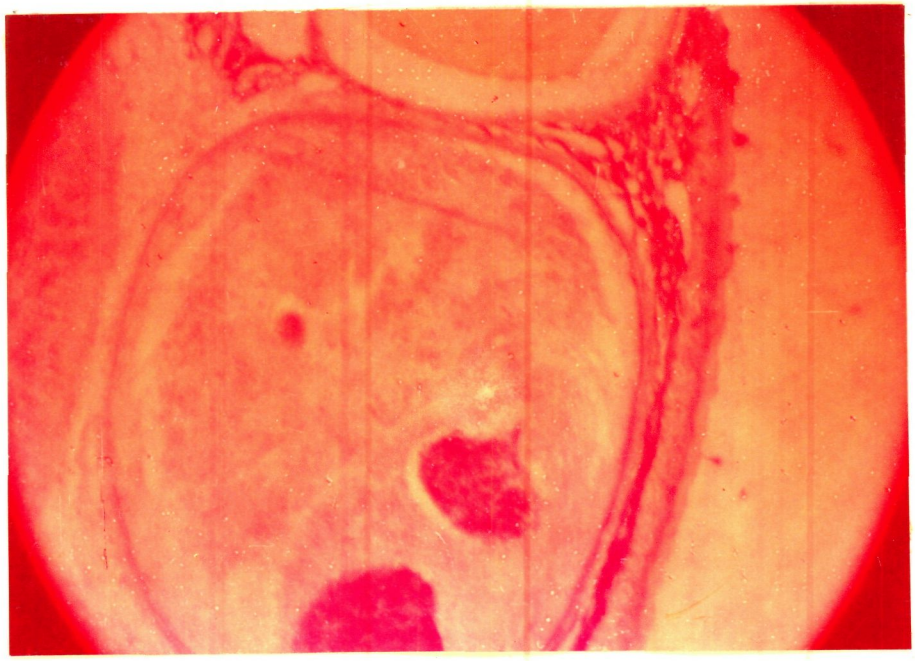


PLATE IX

TABLE. X. 1. T.S. of F. gigantica through anterior parenchyma — Best's Carmine.

2. T.S. of F. gigantica through anterior parenchyma — PAS.

(1)



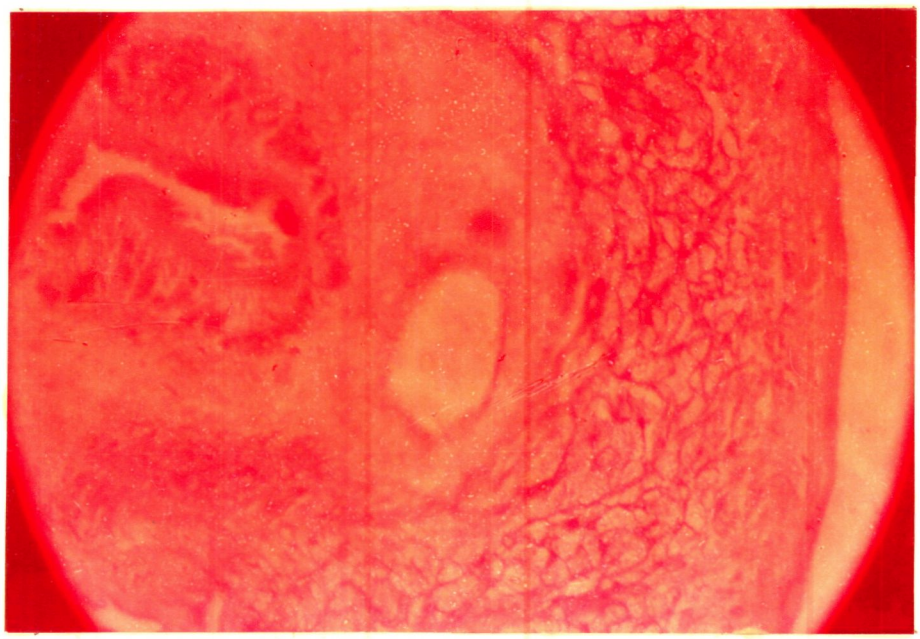
(2)

PLATE X

PLATE. XI. 1. T.S. of *F. gigantica* through cirrus sac — Best's Carmine.

2. T.S. of *F. gigantica* through testis —Mallory's triple stain.

(1)



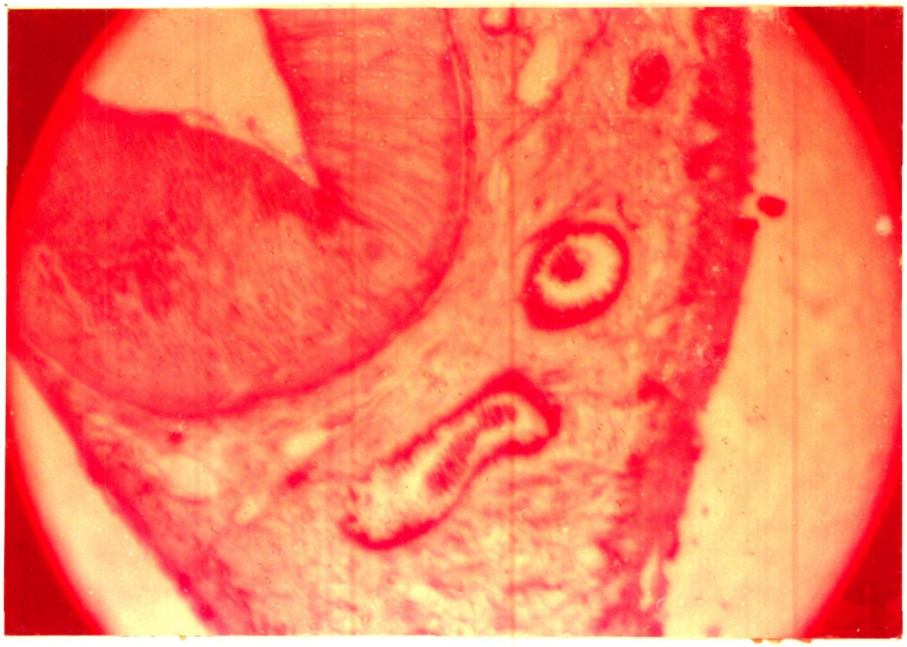
(2)

PLATE XI

TABLE. XII. 1. T.S. of F. gigantica through acetabulum — Heidenhain's azan

2. T.S. of F. gigantica — Acetone Sudan Black B.

(1)



(2)

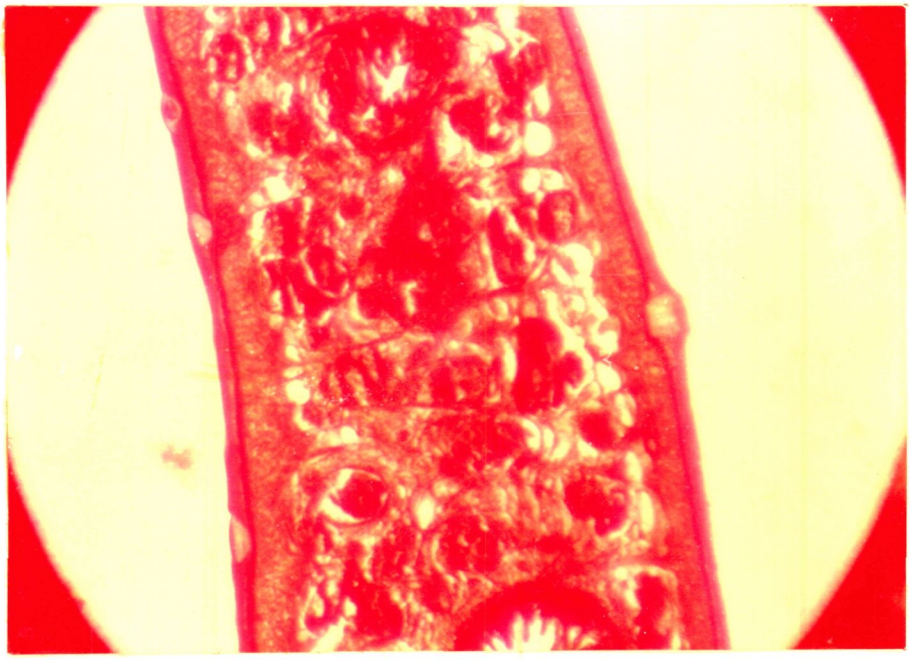


PLATE XII

PLATE XIII.

1. T.S. of *F. gigantica* - Acetone Sudan black B.

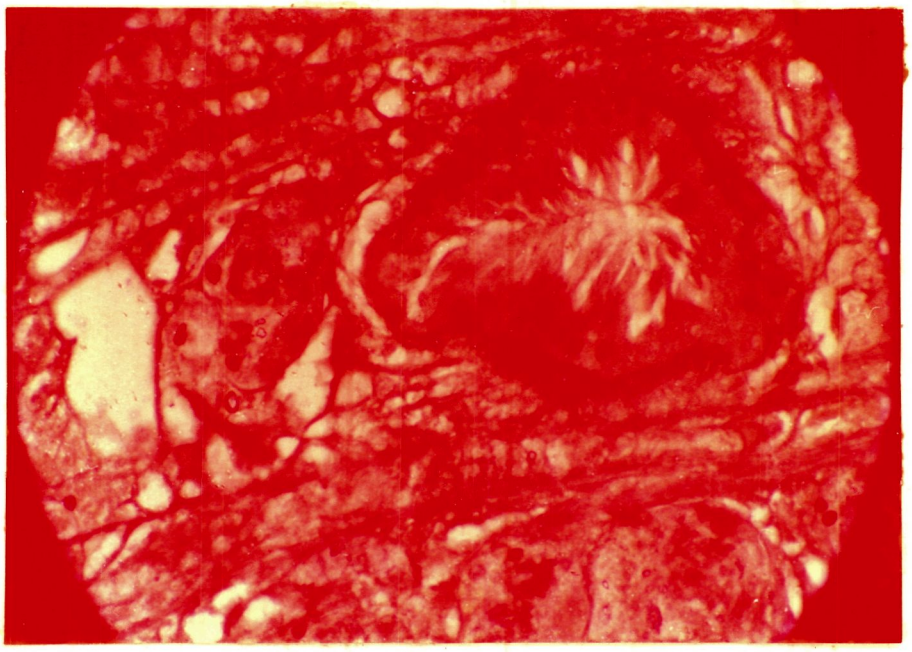


PLATE XIII